NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES (NIAID)

WORKSHOP ON VACCINES FOR HEPATITIS C VIRUS

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Introduction.

The last few years have seen unprecedented growth in research on hepatitis C virus (HCV), a field which had long remained relatively intractable due to the lack of a cell culture system or convenient small animal model. Now, recent breakthroughs are poised to yield a treasure trove of new knowledge and research and clinical tools. Subgenomic HCV replicons paved the way for understanding replication and provided a platform for drug discovery systems. A new chimeric SCID mouse model, using transplanted human liver tissue, opened the door for experimental infection of human liver tissue. And, most exciting of all, a fully permissive cell culture system is finally available that provides for the assembly and release of infectious virus particles. Meanwhile, creative and elegant studies using chimpanzees - the only available infectious animal model – have been providing critical immunologic insights into HCV persistence and clearance.

More than 170 million individuals worldwide (~3% of the global population), are chronically infected with HCV and at risk for severe progressive inflammatory liver disease leading, in some cases, to cirrhosis and hepatocellular carcinoma. Without effective drugs or a vaccine, persistent HCV infection will continue to take a substantial toll on global health. Thus, this first Workshop on Vaccines for Hepatitis C Virus was long overdue. About 50 scientists from academic institutions, NIH, CDC, FDA, and industry discussed the spectrum of issues ranging from epidemiology to vaccine strategies, clinical trials and regulatory concerns. Many points of consensus emerged, while several other issues attracted more controversial and dissimilar opinions. The following narrative reflects the discussions at that Workshop.

WHY A VACCINE NOW AND WHO WILL IT BENEFIT?

The question is rightly asked as to why we need a vaccine now. The availability of new and effective tests for HCV has all but eliminated the most significant route of HCV transmission – blood transfusion. However, a not insignificant population at high risk is the intravenous drug users (IDU), a relatively young group numbering almost 2 million in the U.S alone, about half of them African Americans. Given the unpredictable nature of HCV infection in IDU and the serious health consequences of HCV infection, it seems likely that a preventive HCV vaccine may initially be restricted to IDU and other high risk groups such as health care workers, followed by wider adoption as a common vaccine of childhood or adolescence. Indeed, limited use of the hepatitis B virus vaccine in small at risk populations paved the way for recommending its universal use.

The purpose of HCV vaccines, however, would not be limited to the prevention of transmission of HCV or the establishment of chronic infection. Prevention of long term disease consequences in chronically infected individuals is an important goal. While it is uncertain whether or how so-called therapeutic vaccines would work, there are hopes that such vaccines might reduce serious liver disease, and perhaps even reverse fibrosis. In practice, however, such vaccines are challenging to design because a sophisticated understanding of the immunological mechanisms at play in chronically infected individuals is lacking. The current perception is that vaccines against HCV will likely have the greatest impact when combined with antiviral therapies that reduce viremia.

IS VACCINE-MEDIATED PROTECTION AGAINST HCV POSSIBLE?

Individuals who spontaneously resolve acute hepatitis C appear to have long-lived immunologic memory that reduces the risk of HCV persistence upon re-exposure to the virus. Moreover, in immune chimpanzees (and probably humans) the magnitude and duration of viremia are substantially reduced in a subsequent infection. Chimpanzees experimentally immunized with HCV envelope glycoproteins are sometimes protected from infection by homologous (same genotype), but not heterologous, HCV strains; nevertheless, vaccinated chimpanzees usually resolve infection after a heterologous challenge. Likewise, humans who clear an initial infection and are re-infected appear less likely to become chronically infected. Therefore, while the prospects for a vaccine capable of producing sterilizing immunity

and preventing infection are unlikely, one that might prevent progression from acute to chronic HCV infection appears plausible. This is good news because acute hepatitis C in humans is generally benign clinically and serious liver disease occurs only when HCV infection persists. While these observations are indicative of the importance of adaptive immunological memory against HCV, specific correlates of protection against viral persistence remain to be established. Current evidence suggests that the ability to prime both humoral and cellular immunity will be critical for a successful vaccine.

HUMORAL IMMUNITY

Chimpanzees have been protected against HCV infection by passive antibody-mediated neutralization of the virus before challenge. Moreover, transfer of immune globulins to animals can delay or alter the pattern of HCV replication. Immunization with recombinant HCV E2 envelope glycoprotein has protected some chimpanzees against apparent infection after challenge with HCV, and protection appeared to correlate with high titers of antibodies to E2. Nevertheless, antibodies mediating protection are not well characterized and efforts to translate these observations into vaccine strategies that elicit broadly protective humoral responses face significant hurdles. It is sobering to realize that antibodies do co-exist with viremia in chronically infected individuals, and that their significance to infection outcome has not been thoroughly assessed either in chimpanzees or in humans.

Current use of the term 'neutralizing' often refers to the ability of antibodies to inhibit *in vitro* infection with pseudotyped lentivirus particles bearing HCV envelope proteins. Its correspondence to antibodies that might actually inhibit HCV infection *in vivo* remains to be established. If and when such tests are validated and standardized , the use of pseudotyped viruses offers potential advantages as antibody activities can be assessed against pseudo-particles with HCV envelope proteins of any genotype or defined antigenic variant. More recent progress in pseudo-particle technology has facilitated initial identification of continuous and discontinuous epitopes, the genetic heterogeneity of these epitopes, escape mutations, and studies of their possible relevance to infection outcome. Another surrogate assay is the neutralization of binding (NOB) assay, which measures the ability of serum antibodies (NOB antibodies) to block the binding of recombinant HCV E2 protein to its putative receptor, CD81, on human cells. Again, the clinical

significance of this assay is not clear, nor is its equivalence to the pseudo-particle assays. Within the near future, these surrogate assays will likely be replaced with true virus neutralization assays using cell culture infectious HCV particles produced *in vitro* in fully permissive cell culture systems. Substantial progress has been made in this area since the workshop, and results of such studies will undoubtedly influence vaccine design.

CELL MEDIATED IMMUNITY

There is general consensus that virus-specific cell-mediated immunity is essential for control of HCV infection. This is supported by a well-defined temporal kinetic correlation between initial control of acute phase virus replication and the onset of HCV-specific CD4+ and CD8+ responses. Conversely, antibody-mediated depletion of these T cell subsets from immune chimpanzees that had cleared prior infections results in prolonged or persistent viremia upon re-challenge with the virus. Furthermore, T-lymphocyte responses elicited in chimpanzees vaccinated with non-structural HCV proteins led to substantially better control of acute phase viremia following HCV challenge compared to mock-vaccinated animals.

It is not yet clear why T cell responses succeed in some individuals while they fail in most. The early induction of HCV specific CD8+ and CD4+ T cell responses - targeting a broad array of viral epitopes - correlates with resolution of acute infection in humans, though these responses are not necessarily lacking in chronic infection. However clearance of infection seems to depend more on the durability of these responses than merely early induction or the range of epitopes targeted. The most notable difference between acute resolving and persisting infections is the failure to generate or sustain CD4+ T cell help. This may reflect sub-optimal signaling to CD4+ T cells due to HCV interference with intracellular innate signaling through interferon and/or toll-like receptor pathways. Clearly, better understanding of the role of innate immune responses, which are actively blocked by HCV, in T cell priming will be useful to vaccine development. In addition, T cell control can be subverted through HCV mediated functional silencing of CD8+ T cells, suppression of effector lymphocytes by activation of regulatory T cells and by the selection of virus variants with mutations in MHC class I epitopes. Strategies to restore or enhance innate immune mechanisms that are subverted by HCV proteins may be especially relevant to development of therapeutic vaccines.

CHALLENGES AND OPPORTUNITIES FOR VACCINE DEVELOPMENT

VACCINE STRATEGIES.

Many of the novel vaccine approaches used in HIV vaccine research are now being adapted for vaccination against HCV, which like HIV, is a highly genetically variable RNA virus susceptible to control by cellular immune responses. Approaches for stimulating humoral immune responses have focused mostly on the use of recombinant envelope proteins formulated with adjuvants, while cellular immunity - particularly CD8+ T cell responses- have been generated with particulate protein antigens, viral vectors like recombinant adenoviruses, and plasmid DNA. Prime boost strategies that involve sequential use of recombinant plasmid DNA or viral vectors followed by HCV protein have also been explored. As noted above, recombinant proteins and viral vectors have shown initial promise in preventing or modulating the course of HCV infection in chimpanzees. Therapeutic vaccines that might be used in chronic hepatitis to reduce virus load and retard progression of severe liver disease are very attractive because of the enormous potential for their use, provided safety considerations are satisfied.

VIRUS LOAD AND HETEROGENEITY

Significant questions remain regarding the feasibility of developing vaccines to protect against an RNA virus that has 6 genotypes based on significant nucleotide sequence divergence. HCV diversity and the rapid generation of heterogeneity are therefore considered major factors in the development of persistence, and vaccine design should reflect that concern. It is estimated that 10^{12} virus particles per day are produced in an infected individual. The inherently error prone mechanism of HCV replication thus generates staggering genetic variability, ensuring a high likelihood of immune escape. Some escape mutations may exact a toll on virus replication fitness, forcing reversion to consensus sequences. These considerations raise important practical questions which will influence the selection of virus genes, proteins or peptides as immunogens, and mandate careful selection of optimal sequences (e.g., consensus sequences, ancestral sequences, epitopes where escape diminishes viral fitness), for inclusion in a vaccine. Other issues to be addressed include the

possibility of multivalent vaccines, and whether epitopes that induce humoral or cellular immune escape are better avoided or included in a vaccine.

ANIMAL MODELS FOR TESTING VACCINE IMMUNOGENICITY AND EFFICACY.

Although useful for establishing that vaccines elicit desired immune responses, rodent, rabbit - and sometimes - macaque, models do not always accurately predict immunogenicity in humans, as studies in the HBV and HIV fields have demonstrated. Chimpanzees are the only animal species that can be reliably infected with HCV and thus the only model available for hepatitis C. Despite some important immune differences in HCV induced pathology between humans and chimpanzees, viz., poor antibody responses against the envelope glycoproteins, lower rates of chronic infection, and the absence of severe liver disease (no cirrhosis or hepatocellular carcinoma in the latter), chimpanzees offer unique advantages. The genetic organization and sequences of polymorphic class I and II histocompatibility complex (MHC) genes are virtually indistinguishable between common chimpanzees (*Pan troglodytes*) and humans. Moreover, it is possible to obtain serial liver tissue samples early after experimental infection with a cloned virus to study the evolution of immune response and HCV quasispecies. On the other hand, there are strong arguments for bypassing this animal model in vaccine efficacy studies, particularly for therapeutic applications. The ever decreasing availability of chimpanzees (because of a breeding moratorium), added to the prohibitive cost of vaccinating and challenging with HCV (>$170,000 per animal), makes this an impractical model. Moreover, preclinical testing in chimpanzees prior to human trials for HCV vaccine safety, immunogenicity, or efficacy, is not a specific FDA requirement. Clinical trials to establish the safety and/or immunogenicity of some vaccine candidates have been undertaken in human volunteers who are not HCV infected. Trials of therapeutic HCV vaccine candidates have also been undertaken in chronically infected humans, although these studies are usually approached with caution because of the unknown potential for liver immunopathology caused by cellular immune responses. While there is little on the horizon that can replace chimpanzee as a model for HCV in the near term, GBV-B - a very closely related hepatotropic flavivirus virus that causes hepatitis in tamarins and marmosets - is being developed as a surrogate model. In the meanwhile, the prevailing sense is that the efficacy of prophylactic vaccines should be tested in chimpanzees but not, compulsorily, therapeutic vaccines.

VACCINE EFFICACY TRIALS.

For prophylactic vaccines, the most obvious endpoints are prevention of infection or prevention of persistence of HCV and, for therapeutic vaccines, complete or partial elimination of infection with reductions in the magnitude of viremia. However, unlike HIV, there is no clear relationship between "viral load" (defined usually as the number of viral RNA copies present in plasma or blood) and the severity of liver disease. For therapeutic vaccines, assessing the effect of lowering virus load on the progression of liver disease would likely require clinical trials of impractically long duration. These difficulties are compounded by the possibility that vaccines that reduce but fail to eliminate infection by stimulating immune responses could actually potentiate disease. Although it is not known whether reduction of virus load is a valid surrogate for evaluating clinical improvement of chronic liver diseases, it is currently used as a convenient , easily measurable, and desired endpoint. Perhaps the greatest promise of therapeutic HCV vaccines may be in combination with antiviral therapies, where reductions in virus load in response to drug therapy might favor or synergize with priming of effective immunity. The controversial question of whether vaccines might improve liver histological scores without necessarily terminating or reducing the level of virus replication should eventually be better understood.

Efficacy testing of prophylactic HCV vaccines will require the identification of suitable populations. This will present challenges because in most countries, including the United States, the rates of HCV infection are generally low outside of IDU populations and there are few well-organized IDU cohorts suitable for testing of HCV vaccines. A smaller, special population of interest may be HCV-infected recipients of liver allografts, although immune responses to vaccines would not be normal in such persons. Testing of vaccines in countries with high rates of HCV infection might also be feasible although, again, significant effort in cohort development and clinical trial infrastructure would be required.

VACCINE DEVELOPMENT COSTS, RISKS, AND THE VALUE OF PARTNERSHIPS.

Because of the high cost of development (at a minimum, $500 million), the market for an HCV vaccine is of primary concern. As noted earlier the most obvious target populations for vaccination initially are those at greatest risk for infection. Success might eventually lead to wider use given the serious consequences of HCV infection. The road to completion of successful efficacy trials in humans, particularly for

prophylactic vaccines, will be long and expensive. Uncertainty over mechanisms of immune evasion and lack of an accessible animal model only complicate the task. For therapeutic vaccines, the market comprised of chronic carriers is potentially enormous but this could change in coming years due to very rapid progress in development of specific small molecule antiviral therapies. The development of HCV vaccines would benefit immensely from the establishment of private-public partnerships, like those for other serious and intractable infections like HIV, malaria, and tuberculosis. By providing a pathway to human clinical trials, such partnerships could increase the number of HCV vaccine candidates in research and development pipelines.

NIH Resources

The National Institutes of Health (NIH) are committed to the development and testing of vaccine candidates: http://nihroadmap.nih.gov/publicprivate/

Clinical trials can be undertaken through vaccine and therapy evaluation units (VTEU) funded by the NIAID. Standard polices are in effect governing independent data and safety monitoring, manuscript publication and protection of intellectual property rights. Offices within the NIH offer substantial resources to assist companies navigate with the complex regulatory issues required to move products efficiently into clinical trials, and other resources for their management.
http://www.niaid.nih.gov/dmid/vaccines/develop_vaccines.htm
http://www.niaid.nih.gov/factsheets/vteu.htm